EVALUATION OF MOSQUITOCIDAL ACTIVITIES OF SOME PLANT EXTRACTS ON *AEDES AEGYPTI* (LINN.)

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Abbreviations

VBD	**:** Vector borne diseases
H	**:** hour
min	**:** minute
LC_{50}	**:** 50% lethal concentration
LD_{50}	**:** 50% lethal dose
LC_{90}	**:** 90% lethal concentration
µg/mL	**:** microgram per milliliter
mg/mL	**:** milligram per milliliter
ppm	**:** parts per million
mg/kg	**:** milligram per kilogram
SCS	**:** Solution combustion synthesis
MTT	**:**(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
ZnO	**:** Zinc Oxide
CuO	**:** Copper Oxide
GCMS	: Gas chromatography with mass spectrometry
p-XRD	: Powder X-ray diffraction
FTIR	: Fourier transform infrared spectroscopy
FE-SEM	: Field emission scanning electron microscopy
EDAX	: Energy Dispersive X-Ray analysis
b. w	: Body weight

CHAPTER 1 – INTRODUCTION

Mosquitoes are a significant arthropod vector for diseases like chikungunya, dengue fever, yellow fever, malaria, zika, lymphatic filariasis and the west nile virus. Day by day, pathogens are being introduced into countries where they were previously unknown (World Health Organisation, 2020). Mosquitoes irritate people as well as being a carrier of life-threatening diseases. Mosquito-borne diseases impact approximately 700 million people per year, resulting in over one million deaths (World Health Organisation, 2021). Because of their destructive existence, mosquitoes are regarded as the world's number one enemy (Teklehaimanot and Herath, 1991). Lack of sanitation, a lack of public knowledge, globalization of travel and trade, unplanned urbanization and environmental changes, deforestation and intensive farming (Patel *et al*., 2012) have all had a major impact on disease transmission, resulting in social instability and economic loss for emerging countries (Madhumathy *et al*., 2007; World Health Organisation, 2014).

Mosquitoes are members of the Culicidae family, which comprises over 3,500 species and subspecies. The most dangerous species *Aedes*, which transmits the majority of arboviral diseases. The two most common disease vectors are *Aedes aegypti* and *Aedes albopictus*. These are small insects with a slender segmented body, two halters, two arms, three pairs of legs and elongated mouthparts. *Aedes* species are distinguished by their dark body markings with white lyre-shaped markings and banded legs (Zettel and Kaufman, 2009). Because of its noxious or nasty appearance, German entomologist Johann Wilhelm Meigen first named it *Aedes* in 1818 (Steyskal, 1974). *Aedes* spp found all through tropical and sun tropical regions of the world and easily breed in stagnant water near human habitation (Rajashekara *et al*., 2021).

Mosquito-borne diseases are yet to receive a successful vaccine solution. None of the vaccines available were effective in combating vector-borne diseases (VBDs). As a result, researchers are focusing on vector control methods. New and effective approaches are being introduced every day to reduce mosquito borne diseases by targeting the vector. Control method were categorized into physical (non-chemical), chemical, biological and genetical methods (Vitta *et al*., 2018). Synthetic insecticides are non-selective and have unintended consequences on non-targeted organisms, humans and the environment (biomagnification) (Borah *et al*., 2010; Hardin and Jackson, 2009; Sharma, 2001; Vivekanandhan *et al*., 2018). Long-term use of synthetic chemicals causes nervous system hyperexcitation, disrupt endocrine, asthma and wheezing, eye inflammation, prolonged use could lead to corneal damage and liver damage (Mandavgane *et al*., 2005). This has necessitated the quest for and advancement of environmentally friendly, low-cost indigenous vector control methods.

Various research on natural plant products against mosquito vectors has suggested that they may be a viable alternative to synthetic insecticides in the last decade (Promsiri *et al*., 2006; Rajkumar and Jebanesan 2008; Maurya *et al*., 2009; Ghosh *et al*., 2012).

Plant products projected as weapons against number of VBDs. Natural plant compounds are environmentally and toxicologically safer than synthetic compounds. Phytochemicals including alkaloids, tannins, terpenoids, steroids and saponins are effective as larvicidal, ovicidal, pupicidal and insecticidal (Gutierrez *et al*., 2014; Govindarajan and Karuppannan, 2011a; Aciole *et al*., 2011). Plant products are the treasure house of biologically active molecules that can be used to produce a new compound.

Green nanotechnology is one of the advanced fascinating subjects gaining popularity as a new remedy for mosquito control. Nanoparticles are small organic and inorganic particles with a diameter of less than 100 nanometers. Nanoparticles in conjunction with herbal extracts are helping to eradicate larval populations (Jayaseelan *et al*., 2011; Chitra *et al*., 2015; Siddique *et al*., 2018). The nano materials are synthesized by various methodologies, but these method uses chemicals, high pressure, temperature, toxic contents and are non biodegrable substances (Siddique *et al*., 2018). However the use of chemical and physical method have disadvantge. Thus, biogenic method of green sythesis is more appreciable as it is comparatively cheaper, simple, less use of energy, pressure, temperature and free of toxic chemicals. Recently, the solution combustion synthesis (SCS) method is greatly utilised for the synthesis of metal NPs where plant extracts serve as a fuel. SCS method is a self-sustained thermal reaction, where the heat comes from combustion (Kumar *et al*., 2015; Jayappa *et al*., 2020).

In vitro approaches can duplicate *in vivo* processes since they are a straight forward way, for implementing the determinantal effects of extracts and nanoparticles. In order to produce better outcomes, more focus should be given on experimental procedures. Careful preparation is essential to detect the poison nature of plant extracts and nanoparticles. The brine shrimp nauplii is an effective model system for preliminary screening of toxicity (Kachenton *et al*., 2018; Supraja *et al*., 2018). For comparison, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay also used for determining the nature of extracts a variety of well-known cytotoxicity tests were performed using different cell lines. The plant extracts and biosynthesized nanoparticles on NIH/3T3 normal cell lines was evaluated by MTT assay to determine toxicity property (Das *et al*., 2010; Kuete *et al*., 2017).

Only those therapeutic compounds that have shown promise *in vitro* were recommended for *in vivo* study (Uttekar, 2021). Acute oral toxicity refers to the undesirable effects that

occur after a single dose of a chemical administered within 24 h (Saleem *et al*., 2017). The most important for influential factors in toxicity study is the dose of the herbal extracts. The dose which is not determinantal can be recommended (Tiwari *et al*., 2014).

The current study looks at the impact of plant extracts on the *Ae. aegypti* mosquitoes egg, larval, pupal and adult stages. A total twelve plant species from diverse families were selected and evaluated for mosquitocidal activities against the *Ae. aegypti*. The plants used in this study were selected based on previously reported insecticidal effectiveness against mosquitoes and other insects. Other considerations included intensively investigated plants, commercially significant plants, geographical availability and appropriateness of plant parts.

OBJECTIVES

1. To assess the larvicidal activity of plant extracts against *Ae. aegypti* (Linn.) and to determine lethal concentrations (LC_{50} and LC_{90}).
2. Evaluation of preliminary phytochemicals, antioxidant activities of *S. lycopersicum* L. leaves extracts.
3. To evaluate the mosquitocidal effect of selected plant extract on different instars of *Ae. aegypti*, ovicidal, ovi-position and pupicidal activity.
4. Green synthesis and characterization of metal oxide nanoparticles from *S. lycopersicum* leaves extract.
5. To evaluate *in vitro* toxicity of *S. lycopersicum* extracts and nanoparticles.
6. To evaluate *in vivo* oral acute toxicity of *S. lycopersicum* methanol leaf extract on wistar albino rats.

CHAPTER 2 - REVIEW OF LITERATURE

The recently published literature reports are discussed in detail which were relevant to the present study in this chapter. The chapter also deals with the introduction of mosquito identification, synthetic chemicals, natural products, larvicidal, ovicidal and pupicidal activity of latest articles were reviewed. Further, the green nanotechnology and its experimental procedures were discussed. Toxicity on non-target species and oral acute toxicity report were also noted. However, the literature offers no data on selected plant species for larvicidal activity against vector *Ae. aegypti*.

CHAPTER 3 - MATERIALS AND METHODS

Plant selection, collection and preparation of extracts

The plant materials were selected based on medicinal properties and easy accessibility. The herbarium was prepared and deposited with voucher numbers at Department of Applied Botany, Mangalore University, Mangalagangothri. The plant samples were extracted with different solvents using soxhlet extractor, aqueous extract by soaking method and yield % was calculated.

Colonization of Mosquito

A large number of *Ae. aegypti* mosquitoes were required for routine laboratory work; hence mosquito colonization was necessary. The mosquito larvae were collected from Tin Factory, Krishna Reddy Industrial Estate, Bangalore during and maintained for breeding at National Institute of Malarial Research Centre - Field Unit (NIMR-FU), Bangalore, Karnataka state, India. The mosquito colonies were also brought to the Department of Applied Botany, Mangalore University, Mangalagangotri for further research work. The *Aedes* mosquito has four stages of development as shown in **Figure 1**: egg, larva, pupa and adult. It takes about 8-10 days to complete the life cycle from egg to adult. In 2-3 days, pupae mature into adult flying mosquitoes. Mosquito colonies were properly maintained according to standard protocol (Clemons *et al*., 2010).

Bioassays

Larvicidal and Pupicidal activity

The larvicidal activity of crude extracts of each plant was evaluated as per WHO guidelines with minor modifications. The larval mortality was observed and recorded after 24 h interval up to 72 h (World Health Organization, 2005).

Ovicidal activity: The ovicidal activity conducted as described by Elango *et al*., (2009)

Oviposition activity: The oviposition activity was evaluated as described by Rajkumar and Jebanesan, (2009).

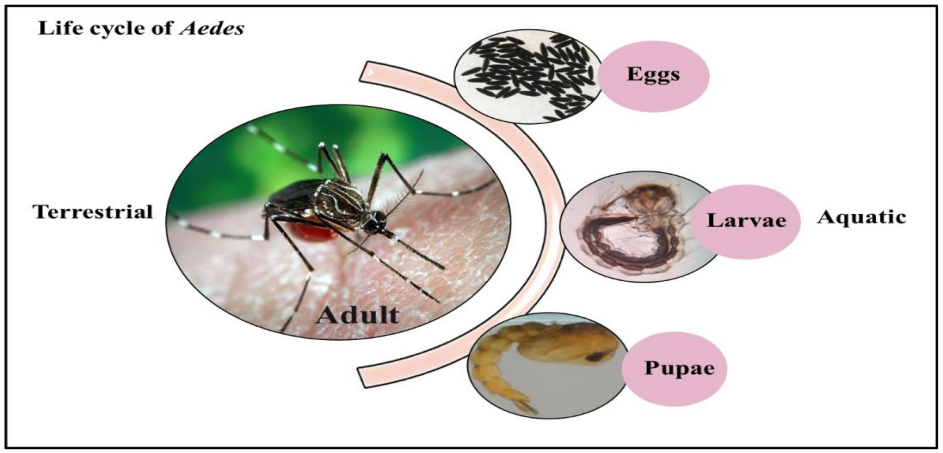

Figure 1: Life cycle of *Aedes aegypti* mosquito

Morphological observations: The morphological modifications of the *S. lycopersicum* treated and control larvae were examined and documented (Warikoo and Kumar, 2013).

Histological observations: Histological examination was performed on the treated and control larvae (Yu *et al*., 2015).

Phytochemical screening of *S. lycopersicum* extracts

Qualitative analysis: The plant crude extract was qualitatively analyzed to identify the presence of various bioactive constituents such as alkaloids, phenolics, flavonoids, glycosides, terpenoids, fixed oils and fats, saponins, gum and mucilage as described by Sasidharan *et al*., 2011; Banu and Cathrine, 2015.

Quantitative analysis

Alkaloids: The total alkaloid content was estimated as a percentage of dry powder (Mir *et al*., 2016).

Saponins: The saponins were quantitatively analysed according to Mir *et al*., (2016). The total saponins were calculated in percentage of dry powder weight.

Phenolics: The total phenolic content of *S. lycopersicum* leaves crude extracts were estimated by the Folin-ciocalteau method. The total phenolic content of extracts absorbancewas measured at 765 nm in triplicates and was expressed as milligrams of gallic acid equivalent pergram of extracts (mg GAE/g) (Sheik and Chandrashekar, 2014).

Flavonoids: The total flavonoids content was determined by the aluminum chloride method. Extracts and standard solutions were checked for absorbance at 510 nm against a blank in triplicates. The total flavonoid content of the extract was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g) (Sheik and Chandrashekar, 2014).

Antioxidant activity: Antioxidant activity of extracts were analysed by reducing power and DPPH (1,1-diphenyl-2-picrylhydrazyl) assay with ascorbic acid as the standard reference (Sheik and Chandrashekar, 2014).

GCMS analysis: GCMS analysis was carried out at Centre for Analytical Instrumentation Kerala (CAI-K), Thrissur, Kerala. The crude extracts of *S. lycopersicum* leaves were analysed *via* Shimadzu GCMS (Model Number: QP2010S) equipped with a Rxi-5sil MS column (30 m x 0.25 mm x 0.25 m film thickness). Compound identification was made based on the comparison of retention indices, area peak percentage using National Institute of Standards and Technology (NIST) 11and WILEY standard mass spectrum 8 libraries (Chalannavar *et al*., 2011).

Synthesis of nanoparticles: Larvicidally active plant was further used for green synthesis

of nanoparticle. Metal oxide nanoparticles (MO NPs) (ZnO and CuO) were synthesized by SCS method. The formed nanoparticles were morphologically and structurally characterized (Ramesh *et al*., 2014)

Characterizations: The biosynthesized nanoparticles were analyzed for its morphology, purity and crystallinity. Different characterizations were performed to confirm the synthesis of nanoparticles *via* UV-Visible spectrophotometry, FTIR, FE-SEM attached with EDAX and p-XRD.

***In vitro* toxicity studies**

Brine shrimp lethality assay: Brine shrimps (*Artemia salina*) toxicity assay was used to determine the lethal nature of *S. lycopersicum* extracts and biosynthesized nanoparticles as described by Nondo *et al*., (2011).

Cytotoxicity: The MTT assay was carried out at Cytxon Biosolutions Pvt. Ltd., Hubli, Karnataka. Mouse embryonic fibroblasts (NIH/3T3) cell lines were used for determining the cytotoxicity. The viability of cells was calculated as a percentage (Ahmed *et al*., 2019).

***In vivo* acute oral toxicity studies:** *S. lycopersicum* methanol extract (SOLYME) was studied for their *in vivo* toxicity as per OECD guidelines 425 (Saleem *et al*., 2017). The wistar albino rats were purchased from Aditya Biosys Pvt. Ltd., Tumakuru (Reg No: 1868/PO/ReBt/S/16/CPCSEA). Animal care and protocol was approved by Institutional Animal Ethics Committee (IAEC) of Mangalore University, Mangalagangothri, Dakshina Kannada, Karnataka (Reg. No. MU/CARRT/IAECMU-RR7/7/2021-22/9.8.2021).

The test sample was made by dissolving extract in a 2% dimethyl sulfoxide (DMSO) and administering 2000 mg/kg of body weight (Single dose). The animals were humanely sacrificed; hematological, biochemical, organ weight and histological tests were assessed after 14 days of treatment. Later, the LD_{50} was calculated.

Repellence activity: Various test concentrations of extracts (1, 2, 5 and 10%) were prepared for the repellence activity, according to Govindarajan *et al*., 2012.

Statistical analysis: The result of the study was statistically analysed using SPSS 22.00 version. The log-probit analysis was used to calculate the lethal (LC_{50} and LC_{90}) concentration data and other statistics at 95% confidence limits of upper and lower limits. The parameters of animal study data were reported as mean ± standard error of mean (SEM). The differences between the treatment and control groups were examined using one-way analysis of variance (ANOVA) and the multiple comparison determined using Tukey's test. At $p < 0.05$, differences between the groups were considered statistically significant.

CHAPTER 4 - RESULTS

The results are compiled in the fourth chapter to support the thesis objectives. The plant-based vector control is the best approach in minimizing the mosquito population thereby inhibiting the diseases transmission. Plants considered as a boon for treating many diseases and has wide range of phytoconstituents, which are substitute for the synthetic insecticides. Each plant was labelled as mentioned in **Table 1**, along with percent yield and physical character of extracts. Based on the findings, methanol was found to be the most effective solvent for extracting phytoconstituents.

Table 1: List of plants used for larvicidal activity, solvent used, percent yield and extracts nature.

Sl No.	Plant Name	Solvent used	% Yield	Extracts physical nature
1.	*Gymnostachyum febrifugum* Benth. (GYFB)	Hexane	2.8	Dark brown, sticky nature, pungent odour
		Methanol	8.8	Dark green sticky and no odour
		Aqueous	13	Dark brown with a stinking smell
2.	*Centratherum punctatum* Cass. (CEPU)	Hexane	3.6	Light green with good aroma, sticky
		Methanol	13.9	Dark, powder form without any essence
		Aqueous	9.3	Brown colour powder with an aroma
3.	*Paederia foetida* L. (PAFO)	Hexane	6.0	Dark green, stick with strong pungent smell
		Methanol	13.2	Dark sticky nature, absence of pungent
		Aqueous	8.0	Dark brown
4.	*Salvadora persica* L. (SAPA)	Hexane	7.85	Dark seaweed green with aroma and sticky
		Ethyl acetate	8.6	Sticky with dark colour and no smell
		Methanol	17.2	Powder with Light brown colour
		Aqueous	11.87	Little sticky with dark brown colour and no aroma
5.	*Careya arborea* Roxb. (CABA)	Petroleum ether	3.0	Dark colour with stick nature and stifling
		Methanol	15.4	Dark chocolate colour with sticky nature with no aroma
		Aqueous	8.2	Dry powder with algal colour no odour
6.	*Cleome gynandra* L. (CLGY)	Petroleum ether	6.22	Dark green with sticky nature
		Methanol	14.20	Dark green with sticky nature
		Aqueous	11.57	Powder with leaf green colour
7.	*Solanum lycopersicum* L. (SOLY)	Petroleum ether	7.78	Dark green, sticky with astringent
		Methanol	23.84	Dark green colour and slightly semi-solid
		Aqueous	13.0	Powder in brown colour without odour

8.	*Cascabela thevetia* (L.) Lippold. (CATE)	Petroleum ether	3.8	Dark with sticky nature
		Acetone	5.4	Dry extract in a dark colour
		Methanol	19.2	Dark brown with pungent
		Aqueous	11.1	Brown powder
9.	*Abutilon hirtum* (Lam.) (ABHI)	Petroleum ether	5.6	Sticky with dark colour and no smell
		Acetone	7.8	Dark green colour and slightly semi-solid
		Methanol	16.44	Semi liquid with dark colour
		Aqueous	12.97	Dark brown with slightly sticky
10.	*Cassia javanica* L. (CAJA)	Petroleum ether	4.7	Dark brown colour with no odour
		Methanol	16.8	Semi-solid extract with brown colour
		Aqueous	8.0	Dry powder in dark brown
11.	*Cicer arietinum* L.(CIAR)	Hexane	5.0	Dark brown with sticky
		Methanol	10.86	Dark brown colour with no odour
		Aqueous	5.0	Dry powder in green colour
12.	*Psidium guajava* L. (PSGY)	Hexane	2.2	Dry sticky dark green colour with a nice odour
		Methanol	13.2	Green colour and in powder form with the aromatic smell
		Aqueous	4.56	Dry powder dark brown with a pleasant smell

Preliminary screening of larvicidal activity (LA) against *Ae. aegypti*

The LA of the selected plants was tested against a laboratory strain of 3rd instar *Ae. aegypti*. Twelve plant extracts were screened individually for preliminary larvicidal activity against *Ae. aegypti* for 48 h with different solvent extracts. Among them, seven plant extracts were potential active against *Ae. aegypti* larvae. The highest larvicidal efficacy was found in a methanol extract of *S. lycopersicum* followed by followed by *C. gynandra*, *A. hirtum*, *P. guajava*, *C. thevetia*, *C. arborea* and *S. persica*. *G. febrifugum*, *C. punctatum*, *P. foetida*, *C. javanica* and *C. arietinum* were not effective against *Ae. aegypti* larvae even after 72 h of treatment. **Figure 2** depicts the larvicidal activity of plant extracts and its effect on *Ae. aegypti* larvae. The lethal concentration values (LC_{50}) of plant extracts vary from 20.323-263.02 mg/mL. $p < 0.05$ considered as significant data. As a result, *S. lycopersicum* was recognized as an active plant to fight against *Ae. aegypti*.

Morphological observations

Under a stereomicroscope, the treated larvae show morphological alterations such as coiling, abnormal movement, deformed body parts, oozing out of digestive contents and shrunken body.

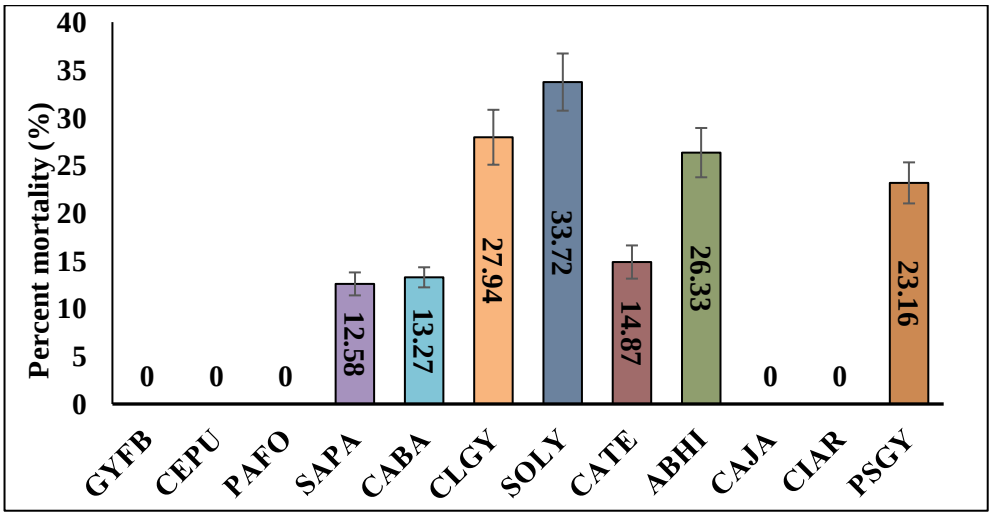

Figure 2: Data are expressed as mean±SEM in percentage mortality of plants against 3rd instar larvae of *Ae. aegypti* at 48 h. GYFB - *Gymnostachyum febrifugum,* CEPU - *Centratherum punctatum,* PAFO - *Paederia foetida,* SAPA - *Salvadora persica,* CABA - *Careya arborea,* CLGY - *Cleome gynandra,* SOLY - *S. lycopersicum,* CATE - *Cascabela thevetia,* ABHI - *Abutilon hirtum,* CAJA - *Cassia javanica,* CIAR - *Cicer arietinum and* PSGY - *Psidium guajava.*

Histological observations

The *S. lycopersicum* treated and untreated *Ae. aegypti* larvae were further cross sectioned through anterior, thoracic and abdominal regions and observed histological changes at various parts leading to disorganization at cellular level and subsequent deaths.

Larvicidal activity of *S. lycopersicum* against different instar of *Ae. aegypti*

According to the findings, the first instar larvae of *Ae. aegypti* are more vulnerable to extracts than the second and third instars. Larvicidal efficacy against different instars revealed significant ($p < 0.05$) results.

Pupicidal activity of *S. lycopersicum* against *Ae. aegypti*

The percent mortality of pupae was high in methanol extract comparison to petroleum ether and aqueous extracts.

Ovicidal and oviposition activity of *S. lycopersicum* against *Ae. aegypti*

The results confirmed that, the ovicidal activity was more in methanol followed by petroleum ether and aqueous extracts with 52.16%, 19.14% and 15.33% against *Ae. aegypti*, respectively. The oviposition activity results confirmed that the extracts of S. lycopersicum was acted as attractant rather than deterrent.

Phytochemical screening

Qualitative analysis

The preliminary phytochemicals analysis of *S. lycopersicum* extracts revealed the presence of alkaloids, phenolics, flavonoids, glycosides, terpenoids, phytosterols, saponins and tannins.

Quantitative estimation

The quantitative results of alkaloids, phenolics, saponins, flavonoids were determined in *S. lycopersicum* extracts. In the present study, the total alkaloid and total saponin contents were found to be 0.086±0.0046 mg/g and 0.438±0.0062 mg/g, respectively. The total phenolics and flavonoids content were determined using gallic acid and quercetin as standard.

Antioxidant activity of crude extracts

Reducing power assay

The reducing power assay was determined using a calibration curve of regression equation i.e. y = 26x-0.0071, the R^2 value of 0.996 and expressed as ascorbic acid equivalent/g.

DPPH radical scavenging activity

A significant difference was observed between the extracts and standard ascorbic acid. The IC_{50} value of methanol, petroleum ether and aqueous extracts were calculated (344.34, 963.25 and 507.48 µg/mL).

GCMS analysis

The crude extracts of *S. lycopersicum* were analysed by GCMS and results revealed a variety of phytoconstituent. The phytol acetate (42.66%), neophytadiene (29.38%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (10.01), methyl palmitate (7.39%) as major and other minor compounds were detected.

FTIR analysis

The functional group of each crude extracts were determined by FTIR analysis. The functional group such as O-H stretch, C-H stretching, C=C stretching, O-H bending, N-H stretching, C-N stretching, C=H bending, C=O stretching, S=O stretching, C=C bending, C-O stretching vibrations was found in the extracts which implies the presence of polyhydroxy compound, alkanes, conjugated alkene, amines, alcohol, amides, unsaturated ketones.

Synthesis and characterizations of Zinc Oxide (ZnO) and Copper Oxide (CuO) NPs using *S. lycopersicum* leaf extract.

The larvicidally active plant was further utilized for the synthesis of bionanoparticles which are effective at preventing mosquito vectors. The synthesized particles were found to be pure, nanosized and ecofriendly. The ZnO and CuO Nps were visually confirmed by the colour changes. After 2 h of calcination in a muffle furnace, white powder formation confirmed the synthesis of ZnO and black powder for CuO (**Figure 3**).

In UV-Visible spectrophotometry analysis, ZnO and CuO Nps revealed a clear absorption band at 353 and 302 nm. The sharp peak in the spectrum suggests that the particles were

nanoscale in size. The FTIR spectra of ZnO Nps, shows the metal oxide (ZnO) stretching vibration peaks at 435 cm^{-1}; 513 $cm^{-1}$and for CuO Nps at 475 cm^{-1}. The extract phytoconstituents serve as a capping, reducing and stabilizing agent. FE-SEM images showed that nanoparticles developed were rod or pellet shaped structure with some agglomeration. EDAX spectra revealed that the produced nanoparticles were elementally pure. powder x-ray diffraction. The average particle size was calculated using the Debye Scherrer equation. The average crystallite size of ZnO and CuO Nps was 40.93 and 14.26 nm, respectively.

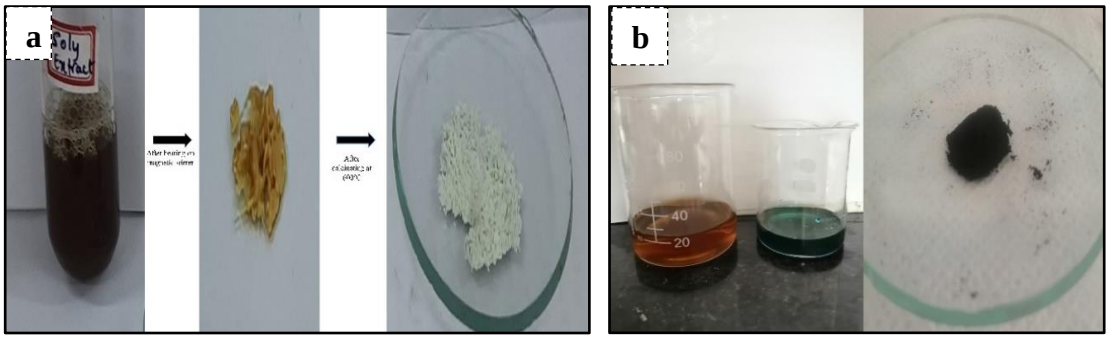

Figure 3: Visual observation of ZnO (a) and CuO (b) Nps from *S. lycopersicum* leaves by SCS method

Larvicidal activity of ZnO and CuO Nps from *S. lycopersicum* leaf extract

After treatment with *S. lycopersicum* nanoparticles, considerable mortality rate was noted in the larvicidal activity. The LC_{50} and LC_{90} values of ZnO and CuO Nps were 12.99, 25.26; 27.79 and 67.76 mg/mL, respectively.

***In vitro* toxicity**

Brine shrimp lethality assay

Extracts and nanoparticles showed toxic effect on brine shrimp nauplii.

Cytotoxicity studies

In cytotoxicity my MTT assay of *S. lycopersicum* extracts were showed the IC_{50} value in methanol (370.00 µg/mL) petroleum ether (283.71 µg/mL) and aqueous extracts (362.43 µg/mL). The results of the cytotoxicity in NIH/3T3 cell line showed IC_{50} <100 µg/mL, 103.07 µg/mL for ZnO and CuO Nps, respectively.

***In vivo* acute oral toxicity**

In vivo results showed that, no mortality was detected in a limit test employing a dose of 2000 mg/kg b.w. of *S. lycopersicum* leaves methanol extract (SOLYME). LD_{50} of *S. lycopersicum* methanol extract was found to be more than 2000 mg/kg. SOLYME treated group revealed changes in behaviour such as somatomotor activity, itching and sleep in the first 30 min of treatment. Vital organs such as the heart, kidney, spleen and liver from testing animals, there was no evidence of injury revealed. The weight of the organs were

recorded and showed no significant significant differences between the treated and vehicle control groups. In hematological parameters, the vehicle control group and treated group showed significant changes ($p < 0.05$) in MCH and neutrophils. In biochemical parameters, creatinine and urea showed no significant changes in vehicle control and extract treated groups. The liver functioning parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase and total protein did not differ significantly between the groups. The present parameter test results are within the safe ranges. The histological analysis of vital organs (heart, liver and kidney) revealed no differences between vehicle control and SOLYME treated group.

Repellent activity

The repellent ability of *S. lycopersicum* methanol extract against blood-starved adult females of *Ae. aegypti*. The plant crude extract protected the test individuals from mosquito bites without causing an allergic reaction. The methanol extract of *S. lycopersicum* has the highest repellency of 60 min against *Ae. aegypti*.

CHAPTER 5 - DISCUSSION

Mosquitoes spread more diseases than any other group of insect species, affecting millions of people each year all over the world. The distribution of these diseases is determined by complex factors such as urbanization, travel and trade all of these changes in the environmental have a significant impact on diseases transmission (World Health Organisation, 2014). Mosquitoes act as a carrier for various diseases such as dengue, chikungunya, zika and other arboviral diseases. About 17% of all infectious diseases spread by mosquito vectors (World Health Organization, 2020; Sahak *et al*., 2020). According to the latest report, a total of 3,541 mosquito species were recorded throughout world, India harbors 404 species accounting for 11.4%. India is geo ecologically suited for vector-borne diseases. Among mosquito species, *Aedes* (*Ae. aegypti* and *Ae. albopictus*) are endemic and invasive species that can adapt easily to the ongoing climatic changes. Furthermore, all viral diseases caused by *Aedes* are passed down in the ovary through a mechanism known as trans-ovarian transmission (Ghosh and Ghosh, 2020).

These fatal diseases have no known cures or vaccination. The only method to protect ourselves against is to avoid mosquito bites. In addition to reducing illness incidences, managing mosquito populations is a critical challenge. Despite the availability of effective chemical techniques, their continued use has affected the biological system, causing resistance and lethal impacts on non-targeted species (Demok *et al*., 2019; Hamid *et al*., 2018).

To address these issues, a new and alternative method for controlling vector borne diseases is required. This has prompted the researchers to develop a new biological agent that is

alternative to existing insecticides and easily degradable. Plants and their by-products have had a wide variety of bioactive compounds that are strong enough to control mosquito's insecticidal property (Ramasamy *et al*., 2012; Vivekanandhan *et al*., 2018; Hayatie *et al*., 2015). Several studies have published ovicidal, larvicidal, pupicidal, adulticidal and insecticidal assays using various extracts from various plant parts (Elango *et al*., 2009; Govindarajan *et al*., 2008, 2011b). Hence, the present study evaluated the larvicidal activity of 12 plant extracts *viz. G. febrifugum*, *C. punctatum*, *P. foetida*, *S. persica*, *C. arborea*, *C. gynandra*, *S. lycopersicum*, *C. thevetia*, *A. hirtum*, *C. javanica*, *C. arietinum* and *P. guajava* against late third and early fourth instar larva of *Ae. aegypti*.

Overall, the results of the study show that some plant extracts were effective to larvae; however, few larvae survive to become adults after being exposed to plant extracts. The LC_{50} values of plants range from 20.323-263.02 mg/mL. *S. lycopersicum* was found to be an effective plant species against *Ae. aegypti* based on lethal concentration (LC) values. The larvicidal mortality was concentration dependent and exposure time. The findings of this research are consistent with those of other studies published in the literature, which show a wide range of results for different mosquito species. Earlier, Kumar, (2012) assessed larvicidal potential against early fourth instar larvae of *Ae. aegypti* using fifteen local plants, where, 100% mortality was observed in only ten plants. Likewise, Hari *et al*., (2018) reported the larvicidal activity of different solvent extracts of four plants against *Ae. aegypti* and found that petroleum ether extract showed higher efficiacy towards dengue vector.

According to preliminary larvicidal studies *S. lycopersicum* methanol extract was dominant against *Ae. aegypti* with 100% mortality at 50 mg/mL after 48 h of exposure. These results are in agreement with several other reports. Molan *et al*., (2016) found that a 100 mg/mL extract of *S. lycopersicum* seed and fruit was successful against *Culex quinquefasciatus*. Gutierrez *et al*., (2014) found that methanolic extract of *Tinospora rumphii* leaf showed 90% larval mortality at 24 h which increased to 93% at 48 h with LC_{50} and LC_{90} values of 10 and 46 mg/mL. The variation in larval toxicity varies due plant phytochemicals varies among the extracts, solvent used, exposure time, concentration variations and phytoconstituents (Ghosh *et al*., 2012; Eukubay *et al*., 2021).

Furthermore, the efficiacy of *S. lycopersicum* leaf extract was assessed at various stages of *Ae. aegypti* lifecycle (Rajmohan and Logankumar, 2011; Carneiro *et al*., 2021). The efficiacy of *S. lycopersicum* leaf extracts were tested on early stages of *Ae. aegypti* life cycle *i. e*., on different instars, pupae and egg (Ova). Among the instars, 1st instar larvae were more susceptible to the extracts compared to other instars (Jayanthi *et al*., 2012). The

strong pupicidal activity was observed in *S. lycopersicum* extracts with an LC_{50} and LC_{90} values of 89.12, 416.86 mg/mL in methanol, 125.89; 214.28 mg/mL in petroleum ether and 117.76; 199.06 mg/mL at 24 h (Hari *et al*., 2018). Similarly, ovicidal mortality was observed when treated with *S. lycopersicum* leaf extracts withan LC_{50} and LC_{90} values in a methanol extract 10.47 and 165.95 mg/mL, respectively. There is an inverse relationship between concentration and percentage of hatching; as concentration rises, the percentage of hatching decreases. Petroleum ether and aqueous extracts were less effective compared to methanol for ovicidal activity.

The active phytochemicals in methanol extract have an effect on mosquitoes growth stages. Histological studies revealed distinct morphological changes, including total damage to the digestive and respiratory tracts, as well as a shrunken head with dark spots. In control larvae, no morphological changes were observed (Ramasamy *et al*., 2021). The current findings show that the leaf extracts of *S. lycopersicum* include a different group of phytochemicals that act as mosquitocidal. The phytochemicals belonging to different groups like alkaloids, tannins, terpenoids, flavonoids, fatty acids, saponins, steroids and glycosides were present in the extracts. The multiple compound present in the extract act as poison to developing mosquitoes. The presence of neophytadiene, phytol acetate, 3,7,11,15-tetramethyl-2-hexadecen-1-ol compounds may have shown toxic effect on larval development (Ravi *et al*., 2018).

Furthermore, the effect of bio nanoparticles synthesized from leaf aqueous extract of *S. lycopersicum* was investigated. Nanotechnology is a relatively new modern technology in the field of vector control that has a significant to daily life. The phytochemicals in the plant act as a reducing agent for nanoparticles. The biosynthesized nanoparticles were also found to have larvicidal efficacy against *Ae. aegypti*. The synthesized nanomaterials were pure with a nanosize of 40.93 and 14.26 nm for ZnO and CuO Nps, respectively while FTIR reports confirmed the reduction of synthesized nanoparticles by phytochemicals (Chiguvare *et al*., 2016).

In addition, brine shrimp larvae were exposed to various concentrations of plant extracts and nanoparticles to assess the toxic effect of plant extracts and nanoparticles. The results showed that the *S. lycopersicum* extracts were more toxic than nanoparticles at higher concentrations. Similarly, the non-toxic nature of extracts and nanoparticles at lower concentrations is supported by NIH/3T3 treated cells.

The acute toxicity of methanol extract of *S. lycopersicum* was studied for 14 days in accordance with OECD guidelines 425. As far as oral toxicity is concerned, more research

is needed to determine the safest dose of compound to maintain clinical symptoms and signs. The results of the acute oral toxicity showed that, significant changes in behavioral pattern were detected within 30 min of dosage. Blood and hematological parameters of MCH and neutrophils showed significant results compared to control group after 14 days and the macroscopic examination of vital organs such as heart, kidney and liver revealed no lesions. Similarly, no alterations were observed in liver, kidney and heart at cellular level of histological study. The current findings are supported by a large number of research (Unuofin *et al*., 2018; Shakya *et al*., 2020; Villas-Boas *et al*., 2021). The *S. lycopersicum* methanol extract had no toxicological effect on animal model of female wistar albino rats (LD_{50}>2000 mg/kg b. w.). As a result, the methanol extract is deemed safe for further research.

CHAPTER 6 - SUMMARY AND CONCLUSION

The thesis investigates the mosquitocidal activity of various plant extracts, as well as green synthesis, characterization and toxicity testing.

The first Chapter begins with an overview of mosquitos, control strategies and the significance of plant extracts, followed by a brief discussion of green nanoparticles and characterization as well as the thesis objectives.

The review of literature relevant to the current investigation is covered in the **Chapter second**. A thorough examination of mosquitocidal activity that were relevant to the study was included in the literature review. Green nanotechnology, experimental methodologies, characterization, toxicity on non-target species, as well as an oral acute toxicity study were also considered. The literature review provided the framework and recommendations for the scope of the current investigations scope.

The materials and methods used in the study, such as extraction, bioassay approaches, green synthesis and toxicity procedures were discussed in **Chapter three**. The UV-Visible spectrophotometry, FTIR, FE-SEM, EDAX, GCMS and p-XRD were all used in the current study.

Chapter four summarizes the whole findings of the current investigation, from plant extraction yield to preliminary screening of extracts on mosquitocidal efficacy and toxicity. Methanol was shown to be the most effective solvent for extracting phytoconstituents. According to the findings, the plant methanol extract of *S. lycopersicum* had the greatest larvicidal efficacy against *Ae. aegypti*. The treated larvae showed significant morphological and histological changes. The first instar of *Ae. aegypti* are more likely to extracts than the second and third instars. The actions of pupicidal,

oviposition and ovicidal activities are dose dependent. The presence of various metabolites in extracts could account for their mosquitocidal effect. According to the primary phytochemical analysis, the extracts contained alkaloids, phenolics, flavonoids, glycosides, terpenoids, phytosterols, saponins and tannins. GCMS was used to identify the bioactive chemicals and FTIR was used to assess the functional group.

Solution combustion synthesis method was employed to create green metal nanoparticles (ZnO and CuO NPs) from *S. lycopersicum* leaves. The formation of nanoparticles was confirmed by the color change followed by calcination and were characterized. The morphology, shape and size of the nanoparticles were validated using characterization techniques. The larvicidal activity of NPs revealed was found to be significant for *Ae. aegypti*.

The toxicity of *S. lycopersicum* extracts and MO NPs against non-target species *in vitro* was effective but not lethal to NIH/3T3 cell lines. *In vivo* results showed that there was no mortality in a limit test using *S. lycopersicum* leaves methanol extract at a concentration of 2000 mg/kg b.w (SOLYME). The LD_{50} of *S. lycopersicum* methanol extract was determined to be over 2000 mg/kg. The methanol extract of *S. lycopersicum* has the highest repellency against *Ae. aegypti* lasting for 60 min. The findings clearly demonstrated that all of the activities were dose and time of exposure dependent.

Finally, in **Chapter five**, the results of mosquitocidal activity, green synthesis, characterization methodologies, *in vitro* and *in vivo* toxicity and their justifications are discussed in detail.

Finally, *S. lycopersicum* leaf extracts were found to be highly effective against *Ae. aegypti*, a lethal mosquito species. As a larvicidal, ovicidal, pupicidal agent, phytochemical components such as phenolics, alkaloids and terpenoids can function together or independently to kill mosquitoes. The extracts contain a high concentration of phenolics, flavonoids and demonstrated potential antioxidant activity. This provides useful information for generating a novel medical benefit from *S. lycopersicum* extract. for developing a new medicinalbenefit from the extracts of *S. lycopersicum*.

Non-target species and NIH/3T3 cell lines were considerably non-toxic at low concentrations. Similarly, *S. lycopersicum* extract had no toxicity in wistar rats. As a result, *S. lycopersicum* could be a promising candidate for developing plant based mosquitocidal agent against *Ae. aegypti* and it could potentially help vector control in future. Furthermore, research on isolating active compounds from *S. lycopersicum* against mosquito larvae is needed for better understanding the mechanism of action of active principles against mosquito larvae.

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LIST OF PUBLICATIONS

- **Nityasree, B. R**., Chalannavar, R. K., Ghosh, S. K., Divakar, M. S. and Sowmyashree, K. (2020). Effect of *Solanum lycopersicum* leaf extracts against larvicidal activity of *Aedes aegypti* L. ***Biomedicine***, *40*(4), 467-473.
- **Nityasree, B. R**., Chalannavar, R. K., Kouser, S., Divakar, M. S., Gani, R. S., Sowmyashree, K. and Malabadi, R. B. (2021). Bioinspired synthesis of zinc oxide nanoparticles by using leaf extract of *Solanum lycopersicum* L. for larvicidal activity of *Aedes aegypti* L. ***Advances in Natural Sciences: Nanoscience and Nanotechnology***, *12*(1), 015009.

NATIONAL AND INTERNATIONAL CONFERENCES

Young Scientist Award

- Oral presentation on "Evaluation of larvicidal activity of some plant extracts against *Aedes aegypti* (Linn.)" in e- International Conference on topic "Plant Biodiversity and Environmental Conservation (ICPBEC-2021)" organized by PG Department of Botany in collaboration with Punjab Biodiversity Authority, Government of India held on May 20th and 21st, 2021 held at Hans Raj Mahila Maha Vidyalaya, **Jalandhar**.

Best Poster Presentation Award

- Poster Presentation on "Chemical Composition and Larvicidal Activity of Essential Oils Extracted from *Psidium* sp. against Chikungunya causing mosquito *Aedes aegypti* L. (Diptera: Culicidae)" in International conference on Medicinal Plants and Drug Discovery on 18th-20th July 2018 in **Bangalore**.

- Oral presentation on "Preliminary Screening of Larvicidal activity of some medicinal

plants against Chikungunya transmitting mosquito, *Aedes aegypti* Linn. (Culicidae)" in International conference in 55th Annual Scientific Conference of the Malaysian Society of Parasitology and Tropical Medicine on 13th and 14th March 2019 in **Kuala Lumpur, Malaysia**.

- Poster Presentation on "Preliminary studies on phytochemicals, *in-vitro* antioxidant and larvicidal activities of *Careya arborea* Roxb. fruit extract on chikungunya transmitting mosquito *Aedes aegypti*" in National Conference organized on 6th and 7th October 2017 by Department of Studies in Biochemistry, Mangalore university, Post graduate center, **Chikka Aluvara**, **Kodagu**.
- Poster Presentation on "Preliminary studies on screening of phytochemicals and larvicidal activity of *Centratherum punctatum* Cass. on chikungunya transmitting mosquito *Aedes aegypti*" in National Conference organized by Mangalore University of Science and Technology in association with The Indian Science Congress Association (ISCA) on 8th and 9th September 2017, **Mangalagangothri**.
- Participated in International Conference on "Malaria in Global Health" conducted on 17th and 18th March 2017 by K.S. Hegde Medical Academy, Nitte University, Deralakatte, **Mangaluru**.
- Participated in XI Joint Annual Conference of ISMOCD and IAE held on topic Public Health in Digital India and Swachh Bharat on 10th-12th June, 2016 in **Bangalore**.

WORKSHOPS

- Participated in National workshop on "Data Analytics Using SPSS" held on 26th and 27th September 2019 at Mangalore University, conducted by IBM and Nihon Communication Solutions Pvt. Ltd. **Bangalore**.

Signature of the Candidate

Signature of the Research Guide